Antimicrobial Activity of Centaurea Species

Kiymet Güven¹, Sezgin Çelik², and İsmet Uysal²

¹Anadolu University, Faculty of Science, Department of Biology, Eskişehir, Turkey; ²Çanakkale Onsekiz Mart University, Faculty of Science and Arts, Department of Biology, Çanakkale, Turkey

Abstract

The antimicrobial activity of the ethyl acetate, acetone, chloroform, and ethanol extracts from the Centaurea species C. ptosomipappoides Wagenitz, C. odyssei Wagenitz, C. ptosomipappa Hayek, C. amonicola Hub. Mor., and C. kurdica Reichardt (Compositae) were investigated by agar-well diffusion assay, and all of the extracts exhibited an antimicrobial effect against most of the bacteria and all of the yeasts tested. Although the plants and extracts differed in their activities against the microorganisms tested, the extracts displayed no antifungal activity against the fungi tested. Ethyl acetate extracts showed most significant inhibitory activity, and the yeasts were more susceptible to the extracts than the bacteria in general. Therefore, minimal inhibitory concentration (MIC) of only ethyl acetate extracts of the samples was determined for some bacteria and the yeasts. In conclusion, C. kurdica was the most active antimicrobial plant. Ethyl acetate extract of C. odyssei and C. kurdica should be further evaluated against human pathogenic yeast isolates, as its antimicrobial activity is stronger than the standard antibiotic ketokonazole.

Keywords: Antimicrobial activity, C. amonicola, C. kurdica, C. odyssei, C. ptosomipappa, C. ptosomipappoides, endemic.

Introduction

Green plants represent a reservoir of effective chemotherapyides and can provide valuable sources of natural antimicrobials (Balandrin et al., 1985; Satish et al., 1999). Plant extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). In particular, the antimicrobial activity of plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (Hammer et al., 1999). Antimicrobials of plant origin are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Kokosha et al., 2002).

The indiscriminate use of antibiotics resulted in the emergence of a number of resistant bacterial strains, and the antimicrobial compounds from plants may inhibit bacteria by a different mechanism than the currently used antibiotics and may have clinical value in the treatment of resistant microbial strains (Elof, 1998). On the other hand, research in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as an alternative solution to health problems in cities. Therefore, with the increasing acceptance of traditional medicine as an alternative form of health care, the screening of plants for active compounds has become very important.

Many species of the genus Centaurea L. (Asteraceae) have traditionally been used for the treatment of various ailments (Kumarasamy et al., 2003). The genus Centaurea L. has been the subject of many antimicrobial activity studies (Vajs et al., 1999; Yeşilada et al., 1999; Skaltsa et al., 2000; Karioti et al., 2002; Kumarasamy et al., 2002, 2003). This study was conducted to investigate the antimicrobial properties of ethanol, acetone, ethyl acetate, and chloroform extracts of five endemic Centaurea L. species; namely, C. ptosomipappoides Wagenitz, C. odyssei Wagenitz, C. ptosomipappa Hayek, C. amonicola Hub. Mor., and C. kurdica Reichardt, collected from Turkey against both clinical and foodborne microorganisms (bacteria, fungi, and yeasts) by using agar-well diffusion assays. Minimal inhibitory concentration (MIC) of ethyl acetate extracts of samples were also determined against some bacteria.

Accepted: October 6, 2004

Address correspondence to: K. Güven, Anadolu University, Faculty of Science, Department of Biology, TR-26470 Eskişehir, Turkey. E-mail: kguven@anadolu.edu.tr

DOI: 10.1080/13880200590903390 © 2005 Taylor & Francis Ltd.
Materials and Methods

Plant materials and extraction procedure

Five endemic Centaurea taxa of Turkish samples (Davis, 1975; Ekim et al., 2000) were collected from original (typical) localities in Turkey by Çelik and Uysal. C. amanicola Hub. Mor. was collected from Yağlıpınar Mountain, Osmaniye (July 2003; 1900 m), C. kurdica Reichardt was collected from Muş (July 2003; 1920 m), C. odyssei Wagenitz was collected from Kazdağ Mountain, Balıkesir (July 2003; 428 m), and C. ptosimopappa Hayek was collected from Hatay (July 2003; 1932 m), and C. ptosimopappoides Wagenitz was collected from Adana (July 2003; 1938 m). The specimens collected were identified with the help of Flora of Turkey and the East Aegean Islands (Davis, 1975). Voucher specimens were deposited in the Herbarium of the Faculty of Science and Arts, Canakkale Onsekiz Mart University (COMU). Collector numbers were given as Çelik and Uysal. The plants previously were air-dried, and then aerial parts (stem, leaf, flower, and fruit) were ground with the help of a Waring blender. Ground samples (20 g) were extracted with 150 ml of ethyl acetate, acetone, chloroform, alcohol solvent (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment. The extracts were filtered using Whatman filter paper (no. 1) and then concentrated in vacuo at 70°C. The residues were stored in a refrigerator until subsequent use. For the bioassay, the extracts were suspended in the same solvents (ethanol, acetone, ethyl acetate, and chloroform) at a concentration of 100 mg/ml.

Microorganisms

A total of 19 microbial genera belonging to 11 bacteria, 2 yeasts, and 5 mold species were used in this study. Proteus vulgaris NRRL B-123, Salmonella typhimurium NRRL B-4420, Bacillus cereus NRRL B-3711, Microccocus luteus NRRL B-4375, Bacillus subtilis NRRL 744, Aspergillus fumigatus NRRL B-163, Penicillium griseofulvum NRRL B-2300, Mucor mucido NRRL B-1425, and Myrothecium verrucaria NRRL B-1875 were obtained from the USDA Agricultural Research Service (Peoria, IL, USA). Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 6538 T, and Pseudomonas aeruginosa ATCC 27853 were obtained from Ege University, Faculty of Science, Department of Biology (İzmir, Turkey). Aeromonas hydrophila, Listeria monocytogenes, and Klebsiella pneumoniae were obtained from Ankara University, Faculty of Veterinary (Ankara, Turkey). Candida albicans, Candida glabrata, and Cladosporium sp. were obtained from Osmangazi University Medical Faculty.

Antimicrobial activity

Agar-well diffusion assay

The antimicrobial activities of the ethanol, acetone, ethyl acetate, and chloroform extracts were evaluated by means of the agar-well diffusion assay (Rojas et al., 2003; Şahin et al., 2003) with some modifications. Twenty milliliters of the specified molten agar (45°C) was poured into 9-cm sterile Petri dishes. A suspension (100 μl) containing 10^8 cfu/ml bacteria, 10^6 cfu/ml yeasts, and 10^4 spore/ml of fungi was spread on the plates of Nutrient agar (Merck), Mueller-Hinton agar (Oxoid) and Sabouraud dextrose agar (Oxoid, Hampshire, UK) medium, respectively. Once the plates were dried aseptically, 6-mm wells were bored using a sterile cork borer. Extracts (50 μl) were placed into the wells, and the plates were incubated for 37°C for 24 h for bacterial strains, 48 h for yeast, and at room temperature for 72 h for fungi. Chloramphenicol (100 mg/ml) for bacteria and ketoconazole (100 mg/ml) for yeast and fungi were used as standard antibiotics. The tests were carried out in triplicate. Antimicrobial activity was evaluated by measuring zone of inhibition against the test organism.

Microdilution assay

The MIC values were also studied for the microorganisms, which were determined as sensitivity to the extracts in agar-well diffusion assay (Vanden Bergh et al., 1991; Koneman et al., 1997; Zgoda & Porter, 2001). Stock solution of the ethyl acetate extract which gave the best inhibition in the agar-well diffusion test was prepared in dimethylsulfoxide (DMSO; Carlo-Erba, France). Dilution series using sterile distilled water were prepared from 2000 to 3.9 μg/ml in test tubes, which were transferred to 96-well microtiter plates. Overnight grown bacterial and yeast suspensions in double strength Mueller Hinton broth were standardized to 10^8 cfu/ml using McFarland No. 0.5 standard solution. Microorganism suspension (100 μl) was then added into the wells. The last-well chain without microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37°C for 18 h, the first well without turbidity was determined as the MIC. Chloramphenicol was used as standard antibacterial agent, whereas ketoconazole was used as antifungal.

Results and Discussion

The antimicrobial activities of C. ptosimopappoides, C. odyssei, C. ptosimopappa, C. amanicola, and C. kurdica ethanol, acetone, ethyl acetate, and chloroform
experiments against microorganisms examined in the current study and their potency were qualitatively assessed by the presence or absence of inhibition zones and zone diameter (Tables 1 and 2).

The results showed that the extracts of *C. ptosemipappoides* mediated some degree of activity against bacteria and yeasts but not fungi (Table 1). *Escherichia coli* was the only isolate that was not inhibited by any of the extracts of *C. ptosemipappoides*. Ethyl acetate extract showed significant antimicrobial activity (defined as a perfectly clear zone with a diameter greater than the other extracts). Ethyl acetate extract of *C. ptosemipappoides* gave an inhibition zone very close to the standard antibiotic ketokonazol.

The extracts of *C. odyseesi* showed some degree of activity against some bacteria and all yeasts (Table 1). Ethanol extract of *C. odyseesi* did not inhibit the growth of *A. hydrophila*, acetone extract of *C. odyseesi* did not inhibit the growth of *E. coli*, and chloroform extract of *C. odyseesi* did not inhibit the growth of *A. hydrophila*, *L. monocytogenes*, *S. aureus*, *M. luteus*, and *B. subtilis*. None of the fungi showed any inhibition zone against any of the *C. odyseesi* extracts. However, ethyl acetate extracts of *C. odyseesi* exhibited significant antimicrobial activities against all the bacteria tested but, particularly, to *P. vulgaris* and *M. luteus* with a diameter same or greater than standard antibiotic chloramphenicol. The inhibition zone diameter of *C. albicans* and *C. globrata* was 24 mm with ethyl acetate extract, although ketokonazol gave only 17 and 21 mm inhibition zones, respectively.

The extracts of *C. ptosemipappoides* exhibited some degree of activity against some bacteria and yeasts but not fungi (Table 1). Only *A. hydrophila* was active against acetone extract, but chloroform extract did not inhibit the growth of *P. vulgaris*, *B. cereus*, *E. coli*, *A. hydrophila*, *L. monocytogenes*, *S. aureus*, and *M. luteus* completely. However, *C. albicans* and *C. globrata* were inhibited strongly by ethyl acetate extracts of *C. ptosemipappoides*.

The results showed that the extracts of *C. amonicola* showed some degree of activity against some bacteria and yeasts but not fungi (Table 2). Acetone and chloroform extracts of *C. amonicola* did not inhibit the growth of some bacteria, but ethyl acetate extract showed significant antimicrobial activity (defined as a perfectly clear zone with a diameter greater than the other extracts) against the bacteria and the yeasts. *C. albicans* and *C. globrata* had an inhibition zone close to the standard antibiotic ketokonazol.

Antimicrobial activities of *C. kurdica* extracts were the most prominent in activity against all microorganisms tested except fungi (Table 2). Only the chloroform extracts of *C. kurdica* did not inhibit the growth of *P. vulgaris*, *E. coli*, and *L. monocytogenes*. However, the ethyl acetate extract exhibited significant

### Table 1. Antimicrobial activity of *C. ptosemipappoides*, *C. odyseesi*, and *C. ptosemipappoides* extracts against the bacterial, yeast, and fungal strains tested based on the agar well-diffusion method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibitory zone in diameter (mm/sensitive strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. ptosemipappoides</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>12</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Pecillium griseofulvis</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Mucor mucedo</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Cladosporium sp.</em></td>
<td>—</td>
</tr>
<tr>
<td><em>Myrothecium verrucaria</em></td>
<td>—</td>
</tr>
</tbody>
</table>

A, ethanol extract; B, acetone extract; C, ethyl acetate extract; D, chloroform extract.
Table 2. Antimicrobial activity of *C. amonicola* and *C. kurdica* extracts against the bacterial, yeast, and fungal strains tested based on agar well-diffusion method.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th><em>C. amonicola</em></th>
<th></th>
<th><em>C. kurdica</em></th>
<th></th>
<th>Standard antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A  B  C  D</td>
<td></td>
<td>A  B  C  D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>8   8  16</td>
<td>—</td>
<td>10  13 19</td>
<td>—</td>
<td>24</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>9   10 18 9</td>
<td></td>
<td>9   12 21 8</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>13  10 16</td>
<td>—</td>
<td>12  12 22 10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>11  12 19 8</td>
<td></td>
<td>12  14 24 10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>8   — 12</td>
<td>—</td>
<td>8   8 12</td>
<td>—</td>
<td>23</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>8   — 15</td>
<td>—</td>
<td>8   8 19 8</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>11  12 17</td>
<td>—</td>
<td>8   10 25</td>
<td>—</td>
<td>25</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>9   10 16</td>
<td>—</td>
<td>10  11 22 8</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>8   8 27</td>
<td>—</td>
<td>8   10 20 7</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>10  13 20 8</td>
<td></td>
<td>12  16 24 9</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>9   8 12 8</td>
<td></td>
<td>8   9 22 8</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>11  12 17 8</td>
<td></td>
<td>12  15 22 8</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>11  13 18 9</td>
<td></td>
<td>11  15 22 8</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>—   — —</td>
<td>—</td>
<td>—   — —</td>
<td>—</td>
<td>23</td>
</tr>
<tr>
<td>Pseudallescheria species</td>
<td>—   — —</td>
<td>—</td>
<td>—   — —</td>
<td>—</td>
<td>23</td>
</tr>
<tr>
<td>Myrothecium verrucaria</td>
<td>—   — —</td>
<td>—</td>
<td>—   — —</td>
<td>—</td>
<td>30</td>
</tr>
</tbody>
</table>

A, ethanol extract; B, acetone extract; C, ethyl acetate extract; D, chloroform extract.

antimicrobial activity against *L. monocytogenes*, *S. aureus*, *K. pneumoniae*, *C. albicans*, and *C. glabrata*.

No antifungal activity of the extracts of any plant species was observed in our study. However, guaianolides from *C. nicolai* Bald revealed inhibitory activity against many fungi except *Trichoderma viride* (Vajs et al., 1999). Similarly, sesquiterpene lactones from *C. thessala* and *C. attica* (Skaltsa et al., 2000) and *C. deusta* (Karioti et al., 2002) showed great antifungal activity.

Although Kumarasamy et al. (2003) found significant antimicrobial activity of serotonin conjugates from *C. nigra* against penicillin-resistant *E. coli*, we observed no (with *C. ptosomipappoides*) or little (with *C. odyssae*, *C. ptosomipappa*, *C. amonicola*, and *C. kurdica*) antimicrobial activity against *E. coli* in our study.

Ethyl acetate extracts of the samples exhibited a stronger and broader spectrum of antimicrobial activity as compared to ethanol, acetone, and chloroform extracts. Therefore, in the determination of MIC, only the ethyl acetate extracts of five *Centaurea* spp. were used against some bacteria chosen from the agar-well diffusion assay. MIC values of the extracts were between

Table 3. The MIC values of *C. ptosomipappoides*, *C. odyssae*, *C. ptosomipappa*, *C. amonicola*, and *C. kurdica* extracts against the microorganisms tested in microdilution assay (MIC in μg/ml).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Ethyl acetate extract</th>
<th>Ethyl acetate extract</th>
<th>Ethyl acetate extract</th>
<th>Ethyl acetate extract</th>
<th>Ethyl acetate extract</th>
<th>Standard antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. ptosomipappoides</em></td>
<td><em>C. odyssae</em></td>
<td><em>C. ptosomipappa</em></td>
<td><em>C. amonicola</em></td>
<td><em>C. kurdica</em></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>62.5</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>125</td>
<td>250</td>
<td>250</td>
<td>125</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>125</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>125</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>125</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td></td>
</tr>
</tbody>
</table>

MIC, minimum inhibitory concentration.
250 and 62.5 µg/ml as it was observed with the standard antimicrobials (Table 3). The MIC value of *C. amonicola* against *S. aureus* was lower (62.5 µg/ml) than chloramphenicol (125 µg/ml), suggesting that it is more effective.

Based on these results, it is possible to conclude that five species of *Centaurea* L., collected from Turkey exhibited a broad range of antimicrobial activity to varying degrees. Particularly, ethyl acetate extracts of *C. odyssaei* and *C. kurdica* showed significant antibacterial and antican didal activities and could be used as antimicrobial agents in new drugs for therapy.

References


